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***REMARKS***

The Office Action mailed December 3, 2003 has been carefully reviewed and the foregoing amendments are made in response thereto. In view of the amendments and the following remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims.

Applicants respectfully submit that no prohibited new matter has been introduced by the amendments. The specification has been amended to update the status of related applications referenced on page 1. The specification has also been amended to remove the hyperlink on page 8. Claim 1 has been amended to clarify the nature of the claimed fragments. Claims 11, 13 15 and 16 have been amended to cancel reference to SEQ ID Nos. 8 and 10, the subject matter of which is presented in new claims 17-19. Support for the amendments to the claims can be found throughout the specification and claims as originally filed. Entry of the amendments is respectfully requested.

Turning now to the Office Action, the specification has been objected to for including an embedded hyperlink in the amendment to page 8. Applicants have deleted the phrase at issue in the amendments presented above. Therefore, the objection may now be withdrawn.

The Office Action also requested that Applicants update the status of the related US applications referenced on page 1. Applicants have amended the specification accordingly. Therefore, this objection may now be withdrawn.

Claim 1 remains rejected under 35 U.S.C. § 112 first paragraph for allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time of filing. The Office Action asserts that the amended claim is not limited solely to the recited SEQ ID Nos. but would still encompass a large scope of varying species.

Applicants respectfully note that claim 1 was amended above and is now directed to an isolated rpoB nucleic acid fragment of a molecule having a sequence selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 8, 9 and 10, wherein said fragment consists of at least about 100 contiguous bases of said sequence. Accordingly, the amended claim does not read on variants having sequences that lie outside the recited SEQ ID Nos. Withdrawal of the rejection is respectfully requested.

Claims 11-15 remain rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by De Beenhouwer *et al.* (WO 95/33851). According to the Office Action, the De Beenhouwer probes would bind to the recited sequences under the conditions recited in the claim, since the temperature of the conditions equate to room temperature. Applicants respectfully traverse the rejection.

Respectfully, temperature is not the only condition that is important when performing hybridizations under stringent conditions. The stringency of hybridization is also controlled by salt conditions, as may be substantiated by reference to any appropriate laboratory manual. For instance, according to Sambrook *et al.*'s Molecular Cloning: A Laboratory Manual, 2nd Ed., to maximize the rate of annealing of a probe and its target, hybridizations are usually carried out in solutions of high ionic strength at a temperature that is 20-25°C below the melting temperature (Sambrook *et al.*, p. 9.50) (attached hereto).

Further, claims 11-15 have been amended to delete reference to SEQ ID Nos. 8 and 10. Thus, while the polynucleotide probes or primers of claims 11-15 hybridize under stringent hybridization conditions to a mycobacterial rpoB sequence selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, and 9 or its complement, they also must not hybridize to the *M. tuberculosis* sequence of SEQ ID NO: 1 or its complement. De Beenhouwer *et al.* does not specifically disclose any probe or primer that hybridizes under stringent conditions to the recited sequences of the amended claim but also fails to hybridize to SEQ ID No. 1. Reconsideration and withdrawal of the rejection is respectfully requested.

Applicants respectfully note that polynucleotide probes and primers that hybridize under stringent conditions to SEQ ID No. 8 or 10 but not to SEQ ID No. 1 are now recited in new claims 17-19, which include the limitation that the recited polynucleotides hybridize to at least 100 contiguous bases of the recited sequences. De Beenhouwer *et al.* does not specifically disclose any probe or primer that hybridizes under stringent conditions to at least 100 contiguous bases of the recited sequences in the new claims but also fails to hybridize to SEQ ID No. 1, as acknowledged in the Office Action on page 7, in paragraph 6. Accordingly, allowance of the new claims, in addition to the newly amended claims presented above, is respectfully requested.

Claim 1 stands rejected under 35 U.S.C. § 112, second paragraph for allegedly being indefinite for its recitation of "consisting of at least about." Without necessarily agreeing with

the rejection, Applicants believe that the amendment to claim 1 presented above resolves any lack of clarity. Withdrawal of the rejection is respectfully requested.

***Conclusion***

In view of the foregoing remarks, Applicants respectfully request withdrawal of all outstanding rejections and early notice of allowance to that effect. Should the Examiner believe that a telephonic interview would expedite prosecution and allowance of this application, he is encouraged to contact the undersigned at his convenience.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No.50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. § 1.136(a)(3).

Respectfully submitted,

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# Molecular Cloning

## A LABORATORY MANUAL

## SECOND EDITION

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*Cold Spring Harbor Laboratory Press*
*1989*

concentrations of protein are believed to interfere with the annealing of the probe to its target. This quenching of the hybridization signal is particularly noticeable when oligonucleotides or probes less than 100 nucleotides in length are used.

7. In the presence of 10% dextran sulfate or 10% polyethylene glycol, the rate of hybridization is accelerated approximately tenfold (Wahl et al. 1979; Renz and Kurz 1984; Amasino 1986) because nucleic acids are excluded from the volume of the solution occupied by the polymer and their effective concentration is therefore increased. Although dextran sulfate and polyethylene glycol are useful in circumstances where the rate of hybridization is the limiting factor in detecting rare sequences (e.g., northern or genomic Southern blots), they are of no benefit when screening bacterial colonies or bacterial plaques. In addition, they can sometimes lead to high backgrounds, and hybridization solutions containing them are always difficult to handle because of their viscosity. We therefore recommend that dextran sulfate and polyethylene glycol not be used unless the rate of hybridization is very slow, the filter contains very small amounts of DNA, or the amount of radiolabeled probe is limiting.

8. To maximize the rate of annealing of the probe with its target, hybridizations are usually carried out in solutions of high ionic strength (6 × SSC or 6 × SSPE) at a temperature that is 20–25°C below the melting temperature ($T_m$). Both solutions work equally well when hybridization is carried out in aqueous solvents. However, when formamide is included in the hybridization buffer, 6 × SSPE is preferred because of its greater buffering power.

9. In general, the washing conditions should be as stringent as possible (i.e., a combination of temperature and salt concentration should be chosen that is approximately 12–20°C below the calculated $T_m$ of the hybrid under study). The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the probe of interest and then washed under conditions of different stringencies.

10. To minimize background problems, it is best to hybridize for the shortest possible time using the minimum amount of probe. For Southern hybridization of mammalian genomic DNA where each lane of the gel contains 10 $\mu$g of DNA, 10–20 ng/ml radiolabeled probe (sp. act. = $10^9$ cpm/$\mu$g or greater) should be used and hybridization should be carried out for 12–16 hours at 68°C in aqueous solution or for 24 hours at 42°C in 50% formamide. For Southern hybridization of fragments of cloned DNA where each band of the restriction digest contains 10 ng of DNA or more, much less probe is required. Typically, hybridization is carried out for 6–8 hours using 1–2 ng/ml radiolabeled probe (sp. act. = $10^9$ cpm/$\mu$g or greater).

11. *Useful facts*:

    a. The $T_m$ of the hybrid formed between the probe and its target may be estimated from the following equation (Bolton and McCarthy 1962):